

# Thermodynamics and dynamics of the formation of spherical lipidic vesicles

E. Hernández Zapata

*Departamento de Física, Matemáticas e Ingeniería,  
Universidad de Sonora, H. Caborca, C.P. 83600, Sonora, México.*

L. Martínez Balbuena

*Departamento de Investigación en Física, Universidad de Sonora,  
Apartado Postal 5-088, 83190 Hermosillo, Sonora, México.*

I. Santamaría Hólek

*Facultad de Ciencias, Universidad Nacional Autónoma de México.  
Circuito exterior de Ciudad Universitaria. 04510, D. F., México.*

We propose a free energy expression accounting for the formation of spherical vesicles from planar lipidic membranes and derive a Fokker-Planck equation for the probability distribution describing the dynamics of vesicle formation. We found that formation may occur as an activated process for small membranes and as a transport process for sufficiently large membranes. We give explicit expressions for the transition rates and the characteristic time of vesicle formation in terms of the relevant physical parameters.

## I. INTRODUCTION

Phospholipid vesicles have been widely used as model systems for studying the dynamics and structural features of many cellular processes, such as endocytosis [1], exocytosis [2], cell fusion [3, 4], transport and diffusion phenomena [5], and membrane elastic properties [6]. In addition to its importance for basic research in the biological sciences, closed vesicles (liposomes) has been used as vehicles for the encapsulation of macromolecules such as nucleic acids [7, 8] as well as polymers and small molecules [9]. Large enough vesicles can be individually manipulated with a glass micropipette [10, 11], and the vesicle membrane rigidity and, in general, membrane elastic properties can be measured [12, 13]. They have also been used as microreactors useful in the study of chemical reactions in geometrically confined spaces [14, 15]. In general, lipidic vesicles constitute nanocontainer systems ideally suited for the isolation, preservation, control and transport of a small number of molecules.

There is a variety of experimental methods to prepare phospholipid vesicle suspensions, reviewed in [16]. One of the most widely used methods is the hydration of a dry phospholipid film [17, 18],

resulting spontaneously in a population of multilamellar vesicles with a high polydispersity in sizes and shapes. On the other hand, the formation of a unilamellar vesicle usually involves an intermediate structure in the form of a planar bilayer fragment, which is unstable, due to its exposed edges. These small planar bilayers can be grown by detergent depletion, phospholipid precipitation or they can be formed from pre-existing bilayers [16]. It is possible to prepare a population of giant unilamellar spherical vesicles when a dry phospholipid film is hydrated in the presence of an AC electric field [19, 20]. The resulting vesicle radius can be as high as 50 micrometers. A similar effect is exhibited by charged phospholipids. The bilayers ionize upon contact with water and they swell due to the repulsion of the bilayers, leading to the spontaneous formation of unilamellar vesicles [21].

In spite of the large experimental work existing, to our knowledge there is no systematic theoretical model describing the dynamics of formation of a unilamellar spherical vesicle from a small planar membrane. Such a model could be useful for the characterization and control of the vesicle formation process and it could be tested by performing single-vesicle simulations and experiments. For instance, the video microscopy analysis of the closing dynamics of laser-generated transient pores on phospholipid membranes [22] could be very useful in this regard.

In this article, we propose a simple theoretical model for spherical-vesicle formation from a planar membrane, assuming that membrane rigidity and edge tension are the main contributions. We first calculate the free energy cost of vesicle formation and then, using this free energy and the rules of mesoscopic nonequilibrium thermodynamics (MNET), we derive a Fokker-Planck equation governing the evolution in time of a nonequilibrium distribution function that depends on time and the mesoscopic variable characterizing the instantaneous state of the system. Our analysis leads to identify that the ratio between the contour energy to curvature energy determines two main mechanisms of vesicle formation: *i*) An activated process for small values of the energy ratio and *ii*) a transport process for values larger than a critical value of the energy ratio. A detailed analysis of these two cases is performed leading to explicit relations for the vesicle formation rates in the first case and for the characteristic formation time in the second one. Our analysis is complemented with a numerical solution of the Fokker-Planck equation.

MNET has been also used in other nanometric processes where curvature and surface tension effects are the main driving forces, such as matter agglomeration systems; see for example [33]. The effect of linear tension on growth morphologies in 2D has been also studied in [34], where the entropy production has been shown to be the dominant selection mechanism.

The article is organized as follows. Sec. II is devoted to derive the expression for the free energy

cost of vesicle formation by using equilibrium arguments. In section III, we use this free energy to formulate a kinetic model for vesicle formation in terms of a Fokker-Planck equation and to analyze its implications. Finally, in section IV we present conclusions.

## II. THE FREE ENERGY

In this section we formulate a simple model for the free energy associated to a phospholipid membrane in the process of wrapping in order to form a spherical vesicle.

We will assume that in every stage of the process the membrane adopts the form of a spherical bowl as shown in Figure 1. In this process, we will consider two competing energies, one  $F_B$  associated with the bending of the membrane that favours planar membranes and another one  $F_l$  due to the contour of the membrane which favours spherical vesicles.

According to the well established Helfrich theory the free energy of bending per unit area,  $f_B$ , obeying the relation  $F_B = \int f_B dA$ , associated to a local deformation of a membrane is given by [23, 24]

$$f_B = 2\kappa(H - c_0)^2 + \bar{\kappa}K, \quad (1)$$

where  $\kappa$  and  $\bar{\kappa}$  are the bending and the saddle-splay moduli respectively,  $c_0$  is the spontaneous curvature of the bilayer. Here  $H = (1/2)(c_1 + c_2)$  is the mean curvature,  $K = c_1 c_2$  is the Gaussian curvature and  $c_1$  and  $c_2$  are the local principal curvatures of the system. Since we are interested in homogeneous bilayers, then we may assume  $c_0 = 0$ . In our bowl approximation, both principal curvatures are identical and equal to inverse radius of the sphere  $r$ :  $c_1 = c_2 = 1/r$ . Therefore, the bending free energy simplifies to

$$F_B = A \frac{\kappa_b}{r^2}, \quad (2)$$

where  $\kappa_b = 2\kappa + \bar{\kappa}$  and  $A$  is the area of the membrane which will be assumed as constant. The contour free energy has the simple form

$$F_l = \gamma l, \quad (3)$$

where  $\gamma$  is the edge tension and  $l$  is the contour length. The total free energy is the sum of both contributions  $F = F_B + F_l$ . Eqs. (2) and (3) can be rewritten in terms of the angle  $\theta$  (see Figure 1), leading to the following expression for the free energy

$$F(\theta) = 2\pi\kappa_b [1 + \cos(\theta)] + 2\gamma (\pi A)^{1/2} \sin(\theta/2). \quad (4)$$

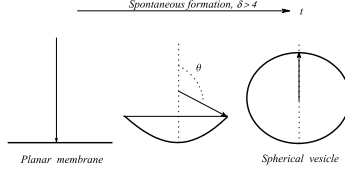


FIG. 1: Schematic representation of vesicle formation from a planar membrane.

To derive this equation, we have used the fact that the total area of the bowl is  $A = 2\pi r^2 [1 + \cos(\theta)]$ , that the contour length is given by  $l = 2\pi r \sin(\theta)$ , and used the trigonometric relation  $\sin(\theta)/[1 + \cos(\theta)]^{1/2} = \sqrt{2} \sin(\theta/2)$ . For convenience, we will use the following dimensionless form of the free energy

$$\tilde{F}(\theta) = \frac{F}{2\pi\kappa_b} = 1 + \cos(\theta) + \delta \sin(\theta/2), \quad (5)$$

where  $\delta = (A/\pi)^{1/2}(\gamma/\kappa_b)$ .

In Eq. (5) it is clear that the parameter  $\delta$  determines the form of the free energy as a function of  $\theta$ , and thus it determines when the planar membrane is stable and when it will spontaneously form a closed vesicle. We can identify the following three regimes (see Figure 2):

1. For  $\delta \leq 2$ , that is, when the linear tension is small compared to the bending constant  $\kappa_b$ , then the free energy has a minimum at  $\theta = \pi$  that corresponds to a planar membrane.
2. For  $2 < \delta < 4$ , there is a competition between contour and bending forces. As a result of this, the free energy has a minimum at  $\theta = 0$  corresponding to a closed spherical vesicle with an energy barrier centered at  $\theta^* = 2 \arcsin(\delta/4)$ . The free energy difference with respect to the planar membrane is given by

$$\Delta\tilde{F} = \tilde{F}(\theta^*) - \tilde{F}(\pi) = \frac{1}{8}(\delta - 4)^2. \quad (6)$$

3. For  $\delta > 4$  there is no energy barrier and the closed spherical vesicles are formed spontaneously.

Let us now estimate the possible values of  $\delta$  for real systems. For lipid bilayers the typical experimental values of the bending modulus are  $\kappa \sim 5 - 25k_B T$  whereas for block copolymer bilayers a typical value is  $\kappa \sim 40k_B T$ , [25, 26]. The bilayer saddle-splay modulus is approximately given by  $\bar{\kappa} \sim -a\kappa$  with  $a \sim 1$  or less [27, 28]. Therefore,  $\kappa_b \sim 5 - 25k_B T$ . The edge tension is of the order of  $\gamma \sim 1 - 2k_B T/nm$ , [25, 26].

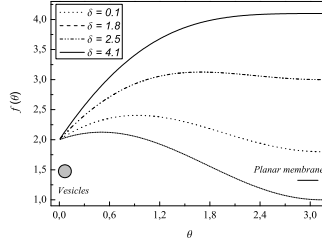


FIG. 2: Dimensionless free energy  $\tilde{F}(\theta)$  as function of  $\theta$  for  $\delta = 0.1, 1.8, 2.8, 4.1$ .

For definiteness let us consider  $\kappa_b \sim 25k_B T$  and  $\gamma \sim 1k_B T/nm$  leading to minimum radius of the vesicles (corresponding to  $\delta = 2$ ) of  $r_{min} \sim \kappa_b/\gamma = 25nm$ . For radius in the range between  $r \sim 25 - 50nm$  an energy barrier has to be overcome in order to form vesicles while for radius larger than  $50nm$  the vesicles will form spontaneously.

### III. DYNAMICS OF VESICLE FORMATION

At isothermal conditions, the free energy given in Eq. (5) can be interpreted (up to a constant) as the energetic cost or the minimum work necessary to form a vesicle

$$R_{min} = 2\pi\kappa_b [1 + \cos(\theta) + \delta \sin(\theta/2)]. \quad (7)$$

This quantity can be used to derive a Fokker-Planck equation for the distribution function  $P(\theta, t)$  of finding the membrane in a stage characterized by  $\theta$  at time  $t$ . This distribution function is normalized and then satisfies a continuity equation of the form

$$\frac{\partial P}{\partial t} = -\frac{\partial}{\partial \theta}(Pv_\theta), \quad (8)$$

where  $Pv_\theta$  is a diffusion probability current in  $\theta$ -space.

The Fokker-Planck equation can be obtained by using the rules of nonequilibrium thermodynamics [29, 30, 31]. This objective can be achieved by first calculating the entropy production of the system using the Gibbs entropy postulate [30]

$$\Delta s = -k_B \int P \ln \frac{P}{P_{eq}} d\theta. \quad (9)$$

In this equation  $\Delta s$  is the entropy change in the process of formation of the vesicle and the integration is carried out over all the range of values of  $\theta$  (from zero to  $\pi$ ). Here,  $P_{eq}$  is the equilibrium reference distribution given by [32]

$$P_{eq} = P_0 e^{-R_{min}/k_B T} = P_0 e^{-2\pi\kappa_b [1 + \cos(\theta) + \delta \sin(\theta/2)]/k_B T}, \quad (10)$$

where  $P_0$  is a normalization factor. When  $\delta < 2$ , Eq. (10) is a very narrow function centered around  $\theta = \pi$ , thus implying that the system remains as a planar membrane. For values of  $\delta$  slightly larger than 2, one finds that the equilibrium state has a coexistence of vesicles and planar membranes. Otherwise, closed vesicles are the preferred configuration of the system.

Now, by taking the derivative of Eq. (9) with respect to time and using Eq. (8), we obtain for the time derivative of the entropy:

$$\frac{\partial s}{\partial t} = k_B \int_0^\pi \frac{\partial}{\partial \theta} (P v_\theta \ln \frac{P}{P_{eq}}) d\theta - \frac{1}{T} \int_0^\pi P v_\theta \frac{\partial \mu}{\partial \theta} d\theta. \quad (11)$$

where we have defined the nonequilibrium chemical potential  $\mu = k_B T \ln |P/P_{eq}|$ . This equation contains two terms, the first one constitutes the entropy flow and the second one is the entropy production  $\sigma$ , given by  $\sigma = (1/T) \int_0^\pi P v_\theta (\partial \mu / \partial \theta) d\theta$ . From Eq. (11) we may formulate linear relationships between the current  $P v_\theta$  and its conjugated force  $\frac{\partial \mu}{\partial \theta}$  in the form

$$P v_\theta = -\alpha P \frac{\partial \mu}{\partial \theta}, \quad (12)$$

where  $\alpha$  is the corresponding Onsager coefficient satisfying Onsager reciprocity relations [29]. This use of a linear relationship assumes that the process is not too far from equilibrium, and therefore it may be not valid in general (this may be the case of charged vesicles). Note that for values  $\delta > 2$ , in the boundary  $\theta = 0$ , the velocity  $v_\theta$  should vanish since the free energy has a local minimum. This condition does not affect the election (12) for other values of  $\theta$ .

Now, by substituting Eq. (12) into (8), and using (10) we finally obtain

$$\frac{\partial P}{\partial t} = \alpha \frac{\partial}{\partial \theta} \left\{ 2\pi \kappa_b \left[ -\sin(\theta) + \frac{\delta}{2} \cos\left(\frac{\theta}{2}\right) \right] P + k_B T \frac{\partial P}{\partial \theta} \right\}. \quad (13)$$

This is the Fokker-Planck equation governing the time evolution of the probability distribution during the formation of the vesicle. It contains a driving term characterized by the force  $\partial F / \partial \theta$  and a diffusion term characterized by the diffusion coefficient  $D = k_B T \alpha$ . Here  $\alpha$  plays the role similar to that of a friction or mobility coefficient in usual Brownian motion. In this case, it can be interpreted as a parameter characterizing the viscous or friction forces exerted on the membrane by the solvent or even it may include interlayer friction [35]. To make sure that  $P(\theta, t)$  will remain confined in the range  $0 < \theta \leq \pi$  the initial condition can be written in the form  $P(\theta, 0) = [H(\theta) - H(\pi - \theta)]P_0(\theta)$  with  $H(\theta)$  the Heaviside function.

#### A. Vesicle formation in the presence of energy barriers

The Fokker-Planck equation (13) can be used to calculate the transition rates  $r_p$  from planar membranes to spherical vesicles (and for the inverse process  $r_v$ ) in the regime where the energy

barrier controls the dynamics, that is, for values of  $\delta$  in the interval  $\delta \in (0, 4)$ . This objective can be achieved by following the usual methods of activated processes [36].

Let us estimate the transition rate  $r_p$  in the stationary state assuming that the number of planar membranes is much larger than the number of vesicles. This can be done by assuming a two-state dynamics in the presence of a barrier. In this case the net current

$$j = -\alpha \left\{ 2\pi\kappa_b \left[ -\sin(\theta) + \frac{\delta}{2} \cos\left(\frac{\theta}{2}\right) \right] P + k_B T \frac{\partial P}{\partial \theta} \right\}, \quad (14)$$

obtained from the Fokker-Planck equation (13), is a constant in  $\theta$ -space, in the stationary case. Then, the transition rate  $r_p$  is defined by  $r_p = j/P_p$ , where  $P_p$  is the total number of planar membranes that can be calculated by integrating the stationary distribution function  $P_s(\theta)$  from  $\theta^*$  to  $\theta = \pi$  [36].

The explicit expression for  $j$  and  $P_p$  can be obtained by expanding in a Taylor series up to second order in  $\theta$  the free energy potential  $F$  about its maximum at  $\theta^*$  and its local minimum at  $\theta = \pi$ . This procedure yields the approximate expressions

$$F_{max}(\theta) \simeq \frac{\pi\kappa_b}{4} (16 + \delta^2) - \frac{\pi\kappa_b}{16} (16 - \delta^2)(\theta - \theta^*)^2, \quad (15)$$

$$F_\pi(\theta) \simeq 2\pi\kappa_b\delta + \frac{\pi\kappa_b}{4} (4 - \delta)(\theta - \pi)^2 \quad (16)$$

To calculate  $j$  and  $P_p$  one then uses Eqs. (15) and (16), respectively. Now, the transition rate  $r_p$  from planar membranes to spherical vesicles is

$$r_p = \frac{\alpha\kappa_b}{4} (4 - \delta)(4 + \delta)^{1/2} e^{-\pi\kappa_b(\delta-4)^2/4k_B T}. \quad (17)$$

This expression is valid as long as the energy barrier is larger than the thermal energy. According to our expression this imposes the condition  $\delta < 4 - \sqrt{\frac{4k_B T}{\pi\kappa_b}}$ . When this condition is not satisfied, the vesicle formation must be analyzed as a transport process. In Figure 3a we show  $r_p$  as a function of  $\delta$  for different values of  $\kappa_b/k_B T$ .

In order to calculate the rate  $r_v$  of the inverse process when the initial condition is such that the number of vesicles is much larger than the number of planar membranes, we first approximate the free energy around the local minimum at  $\theta = 0$ , obtaining

$$F_0(\theta) \simeq \frac{\pi\kappa_b}{4} (16 + \delta^2) - \pi\kappa_b \left( \theta - \frac{\delta}{2} \right)^2. \quad (18)$$

Note that the quadratic term in the approximation of free energy at  $\theta = 0$  is negative. Thus, when evaluating the number of vesicles  $P_v$  around this minimum we obtain

$$P_v = \sigma_v e^{-\frac{\pi\kappa_b}{4k_B T} \delta^2} \int_0^{\delta/2} e^{\frac{\pi\kappa_b}{k_B T} (\theta - \delta/2)^2}, \quad (19)$$

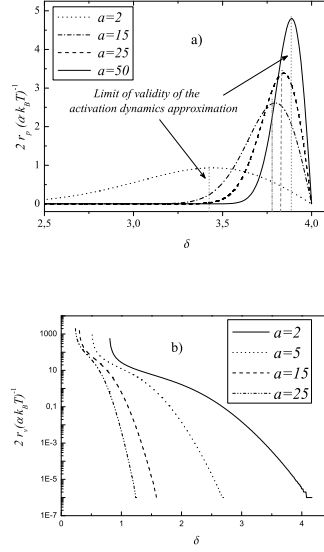


FIG. 3: a) Dimensionless transition rate  $r_p$  as a function of  $\delta$  for different values of  $a = \kappa_b/k_B T = 2, 15, 25, 50$  at room temperature. The vertical lines indicate the limit of validity of the activation dynamics approximation. b) Dimensionless transition rate  $r_v$  as a function of  $\delta$  for different values of  $a = 2, 5, 15, 25$  at room temperature.

where  $\sigma_v d\theta$  is the number of membranes between 0 and  $d\theta$ . In this case, the integration of the Boltzmann factor,  $\exp(-F_0(\theta)/k_B T)$ , must be evaluated between the minimum and the position of the maximum at  $\theta^*$ . For simplicity sake, we have approximated  $\theta^*$  up to first order in  $\delta$ ; that is,  $\theta^* \sim \delta/2$ . After calculating the integral our estimation for the transition rate  $r_v$  from spherical vesicles to planar membranes is

$$r_v = \frac{\alpha\kappa_b}{2} \frac{(16 - \delta^2)^{1/2}}{\text{erfi}\left(\frac{\delta}{2} \sqrt{\frac{\pi\kappa_b}{k_B T}}\right)}. \quad (20)$$

In Figure 3, we show the behavior of  $r_v$  as a function of  $\delta$  for different values of  $a = 2\pi\kappa_b/k_B T$ . For a given value of  $a$ , the transition rate grows as the energy barrier decreases ( $\delta$  increases). For a given value of  $\delta$ , the transition rate depends on the relative value of the bending energy with respect to the thermal energy,  $a$ . For constant temperature  $T$ , decreasing the bending modulus  $\kappa_b$  favours the formation of vesicles associated with increasing values of  $r_v$ . It is interesting to notice that the Arrhenius law is a consequence of two ingredients: i) the assumption of the linear law Eq. (12) which implies a process not too far from equilibrium, and ii) the fact that the minimum of the potential is an extremum, see Eq. (17). This second condition is not fulfilled in the case of Eq. (20) which clearly is not an Arrhenius type law. Non-Arrhenius behaviors can also emerge as



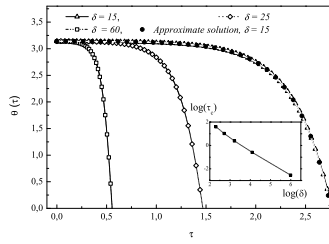


FIG. 4: Angle  $\theta$  as a function of the dimensionless time  $\tau = 2\pi\kappa_b\alpha t$  for the following values of  $\delta$ : 15, 25 and 60 obtained by solving numerically Eq. (21). The solid circles represent an analytical solution given in the text for  $\delta = 15$ . The inset shows a log-log plot of vesicle formation time  $\tau_c$  versus  $\delta$  for  $\delta = 10, 15, 25, 60, 400$ . The slope of the straight line is  $\sim -1.07$ .

a consequence of other mechanisms, see for example [37, 38].

### B. Vesicle formation as a transport process

For values of  $\delta$  larger than 4, the absolute minimum of the free energy occurs at  $\theta = 0$  (corresponding to spherical vesicles) without the presence of energy barriers. Therefore, in this case the dynamics must be analyzed as a transport process.

To do this, one may neglect thermal fluctuations so that the distribution function can be approximated by a Dirac delta function  $P(\theta, t) = \delta[\theta - \theta(t)]$ , [39]. In this case, after multiplying by  $\theta$  and integrating over all  $\theta$ -space, Eq. (13) reduces to the dynamical equation

$$\frac{d}{d\tau}\theta(\tau) = -\frac{\delta}{2}\cos\left(\frac{\theta}{2}\right) + \sin(\theta), \quad (21)$$

where we have defined the dimensionless time  $\tau = 2\pi\alpha\kappa_b t$ . Since this equation cannot be solved analytically, we have solved it numerically by using a Runge-Kutta method. The solutions (open symbols with lines) as a function of  $\tau$  for three different values of  $\delta$  are shown in Fig. 4. As initial condition we used  $\theta(0) = 3.14$  since this value represents a nearly planar membrane but with a small perturbation that permits the membrane to evolve to its equilibrium state (closed vesicle).

As it is clear from the figure, during most of the evolution time the value of  $\theta$  is close to  $\pi$ . Therefore, one may approximate Eq. (21) around  $\theta = \pi$  to first order in its series expansion. This can be solved with the same initial condition leading to  $\theta(\tau) \sim \pi - 0.0016 \exp[(\delta - 4)\tau/4]$ . This solution is represented in Fig. 4 by the solid circles. As can be seen, it constitutes an excellent approximation at all times.

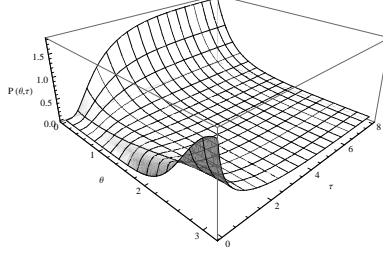


FIG. 5: Probability distribution  $P$  as a function of angle and dimensionless time  $\tau$  obtained by numerically solving Eq. (13) with an initial condition given by a Gaussian distribution centered at  $\theta = \pi$ . At short times ( $\tau < 1$ ) diffusion dominates spreading the distribution. For times  $\tau > 1$  the drifting force dominates and the distribution becomes narrow close to  $\theta = 0$ .

For large values of  $\delta$ , this approximation allows us to estimate the characteristic vesicle formation time  $t_c$ , given by  $t_c = 2/(\pi\kappa_b \alpha \delta) = 2/(\sqrt{\pi A} \alpha \gamma)$ . The inset of Fig. 4 shows that the dependence of  $\log(\tau_c)$  as a function of  $\log(\delta)$  is linear with a slope close to  $-1$ .

We now note that the Onsager coefficient  $\alpha$  is a mobility that depends on the dynamic viscosity of the solvent  $\eta$  and the membrane area  $A$ . Since  $\alpha^{-1}$  has dimensions of energy multiplied by time, it must depend on the combination  $\alpha^{-1} \sim \eta A^{3/2}$ , yielding

$$t_c \sim \frac{2}{\sqrt{\pi}} \frac{\eta A}{\gamma}. \quad (22)$$

This relation predicts that the formation time is given by the ratio between the friction force  $\sim \eta A$  exerted by the solvent on the membrane by the linear tension force  $\gamma$  at the contour of the membrane.

For typical values of vesicle area  $A \sim 10^5 nm^2$ , the dynamic viscosity of the solvent  $\eta \sim 10^{-3} Pa s$  and the linear tension  $\gamma \sim 1 - 2k_B T/nm$ , one obtains that the characteristic formation time is of the order  $t_c \simeq 1 ms$ .

Finally, we have numerically solved Eq. (13) in order to study the effects of thermal fluctuations. We have taken  $\delta = 5$  and  $k_B T/2\pi\kappa_b = 1$ . As an initial condition we have taken a Gaussian distribution centered at  $\theta = \pi$ . At short times ( $\tau < 1$ ) diffusion dominates spreading the distribution whereas for times  $\tau > 1$  the drifting force dominates and the distribution becomes narrow close to  $\theta = 0$ . These results are shown in Fig. 5.

#### IV. CONCLUSIONS

In this article, we proposed a free energy expression accounting for the formation of spherical vesicles from planar membranes. This energy depends on a single state variable and contains two

physical parameters related to the membrane rigidity and to the edge tension. The equilibrium properties of this energy depend on the ratio,  $\delta = (\pi A)^{1/2} \gamma / \kappa_b$ , between the contour energy and the Helfrich curvature energy. When  $\delta < 4$  the free energy presents a barrier which disappears for larger values of  $\delta$ .

Using mesoscopic nonequilibrium thermodynamics rules and the equilibrium information, we have derived a Fokker-Planck equation for the probability distribution describing the dynamics of vesicle formation.

Two cases have been analyzed: *i*) Formation in the presence of barriers ( $\delta < 4$ ) and *ii*) formation as a transport process ( $\delta > 4$ ). In the first case we have derived expressions for the transition rates of formation of vesicles from planar membranes ( $r_p$ ) and viceversa ( $r_v$ ). Our expression for  $r_p$  follows an Arrhenius law [see, Eq. (17)] and is an increasing function of  $\delta$ . The rate of the inverse process  $r_v$  has an unusual dependence on temperature [see, Eq. (20)] due to the fact that the free energy minimum at  $\theta = 0$  (vesicles) is not an extremum. We have found that  $r_v/r_p$  is orders of magnitude smaller than one thus implying that the unwrapping of the spherical vesicles is a very improbable process even in the case when the free energy favours it.

In the second case, the free energy minimum always corresponds to spherical vesicles and can be analyzed by using a deterministic equation for the angle as a function of time after neglecting the effects of thermal fluctuations. A simple analytical expression that is an excellent approximation of the numerical solution allows us to estimate the characteristic vesicle formation time  $t_c$  which is proportional to the membrane area and the viscosity of the solvent, and inversely proportional to the edge tension  $\gamma$ , see Eq. (22). For typical values of phospholipid membranes of linear dimensions  $\sim 100nm$ , we have obtained that  $t_c \sim 1ms$ .

These results suggest that in typical experiments involving video-based measurements, the dynamics of vesicles formation is dominated by a transport process whereas for numerical simulations, in which the studied system is small, the presence of energy barriers could be relevant to the dynamics.

It could be interesting to test the present model by performing single vesicle experiments and simulations in which the detailed evolution in time of the membrane edge can be followed, so that the characteristic vesicle formation time can be obtained.

The proposed model could be useful in the understanding of the mechanisms of phospholipid vesicle formation widely used as model experimental systems to study the thermoelastic properties of cellular membranes.

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